AMENDMENTS TO THE CLAIMS

1-72. **(Canceled)**

73. **(Currently Amended)** A method of determining a definite quantity of a target mRNA encoding a specific sequence in a blood sample comprising:

(a) collecting whole blood;

(b) administering an anticoagulant to the whole blood;

(c) removing erythrocytes and blood components other than leukocytes from the whole blood to yield leukocytes;

(d) lysing the leukocytes with a lysis buffer containing spiked control poly-A RNA to produce a lysate comprising target mRNA and said spiked control poly-A RNA, thereby obtaining amounts of target mRNA and spiked control RNA respectively, wherein said spiked control RNA is non-homologous to RNA from the whole blood;

(e) transferring the lysate to an oligo(dT)-immobilized plate to capture the mRNA;

(f) quantifying the target mRNA and the spiked control RNA, thereby obtaining values of the target mRNA and the spiked control RNA respectively;

(g) determining the percent recovery of said spiked control RNA by dividing the value of said spiked control RNA determined in step (f) by the amount of said spiked control RNA obtained in step (d); and

(h) determining the definite quantity of said target mRNA by dividing the value of said target mRNA determined in step (f) by the percent recovery of said spiked control RNA determined in step (g).

74. **(Canceled)**

75. **(Previously Presented)** The method of Claim 73, wherein step (c) comprises filtration to yield leukocytes on a filter membrane.

76. **(Canceled)**

77. **(Previously Presented)** The method of Claim 73, wherein the anticoagulant is heparin.

78. **(Previously Presented)** The method of Claim 73, wherein the whole blood is frozen and subsequently thawed prior to step (c).

79. **(Original)** The method of Claim 75, wherein the filter membrane is attached to a multi-well filter plate.

80. **(Previously Presented)** The method of Claim 79, wherein 10 to $1x10^{10}$ copies of spiked control RNA are applied to each well of the multi-well filter plate.

81. **(Previously Presented)** The method of Claim 79, wherein $1x10^{5}$ to $1x10^{10}$ copies of spiked control RNA are applied to each well of the multi-well filter plate.

82. **(Previously Presented)** The method of Claim 75, wherein the filter membrane is a polybutylene terephthalate (PBT) fibrous membrane.

83. **(Previously Presented)** The method of Claim 73, wherein step (c) comprises filtration to yield leukocytes on a plurality of filter membranes layered together.

84. **(Original)** The method of Claim 75, additionally comprising washing the leukocytes on the filter membrane with hypotonic buffer to further remove erythrocytes and other blood components.

85. **(Original)** The method of Claim 84, additionally comprising drying the filter membrane.

86. **(Original)** The method of Claim 85, wherein the filter membrane is washed with ethanol and subjected to vacuum aspiration until the filter membrane is dry.

87. **(Original)** The method of Claim 73, wherein the immobilized plate comprises a multi-well oligo(dT)-immobilized plate.

88. **(Original)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises centrifugation.

89. **(Withdrawn)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises vacuum aspiration.

90. **(Withdrawn)** The method of Claim 73, wherein the transfer of lysate to the oligo(dT)-immobilized plate comprises applying positive pressure.

91. **(Previously Presented)** The method of Claim 73, wherein the quantification of target mRNA comprises cDNA synthesis of the target mRNA and amplification of the resulting cDNA.

92. **(Previously Presented)** The method of Claim 79, additionally comprising application of specific antisense primers to each well of the multi-well filter plate during said lysate transferring step.

93-214. **(Canceled)**

215. **(Currently amended)** A method of high throughput quantification of a target mRNA, comprising the steps of:

(a) collecting whole blood;

(b) administering an anticoagulant to the whole blood;

(c) removing erythrocytes and blood components other than leukocytes from the whole blood by filtration to yield leukocytes on a filter membrane;

(d) lysing the leukocytes on said filter membrane with a lysis buffer comprising antisense primers specific to said target mRNA to produce a lysate comprising mRNA comprising said target mRNA with said antisense primers hybridized thereto;

(e) transferring the lysate to an oligo(dT)-immobilized plate to capture the target mRNA;

(f) removing non-hybridized materials from said oligo(dT)-immobilized plate;

(g) adding reverse transcriptase to said oligo(dT)-immobilized plate without addition of further antisense primers, thereby synthesizing cDNA formed by extension of both the immobilized oligo(dT) and the antisense primers,

wherein the cDNA formed by extension of said oligo(dT) remains immobilized to said plate, and the cDNA formed by extension of the antisense primers goes into solution as a result of displacement by the cDNA formed by extension of said oligo(dT) without heat denaturation of said target mRNA and said cDNA formed by extension of the antisense primers; and

(h) quantifying the target mRNA by amplifying the cDNA in said cDNA solution and quantifying the amplified cDNA ~~in~~ from said cDNA solution.

216. **(Canceled)**

217. **(Previously Presented)** The method of Claim 215, wherein a plurality of different antisense primers for different target mRNAs are present in the lysis buffer.

218. **(Currently amended)** The method of Claim 217, wherein each of said different target mRNAs are quantified by quantifying ~~is amplified from~~ the cDNA synthesized ~~formed by extension of~~ from the site of hybridization of the antisense primers to said target mRNA ~~in step (g)~~.

219. **(Previously Presented)** The method of Claim 217, wherein the cDNA solution is removed from the plate and the plate with the immobilized cDNA is stored for future use.

220. **(Withdrawn)** The method of Claim 73, wherein the mRNA quantified is β-actin mRNA.

221. **(Withdrawn)** The method of Claim 73, wherein the mRNA quantified is CD4 mRNA.

222. **(Withdrawn)** The method of Claim 73, wherein the mRNA of a translocation gene involved in leukemia is quantified.

223. **(Withdrawn)** The method of Claim 73, wherein the mRNA of cancer-specific genes from micrometastatic cancer is quantified.

224. **(Withdrawn)** The method of Claim 73, wherein virus-derived mRNA from infected white blood cells is quantified.

225. **(Withdrawn)** The method of Claim 224, wherein the quantified virus-derived mRNA is HIV.

226. **(Withdrawn)** The method of Claim 225, wherein the quantification of HIV mRNA is used to diagnose HIV.

227. **(Withdrawn)** The method of Claim 224, wherein the quantified virus-derived mRNA is CMV.

228. **(Withdrawn)** The method of Claim 227, wherein the quantification of virus-derived mRNA is used to diagnose CMV.

229. **(Withdrawn)** The method of Claim 224, wherein the quantification of virus-derived mRNA is used to monitor blood banks for the presence of viral diseases.

230. **(Withdrawn)** The method of Claim 224, wherein the quantification of virus-derived mRNA is used to study anti-viral drug sensitivity.

231. **(Previously Presented)** The method of Claim 73, wherein the target mRNA is mRNA of apoptosis genes involved in leukemia.

232. **(Previously Presented)** The method of Claim 73, wherein the target mRNA is mRNA of cytokines.

233. **(Currently amended)** The method of Claim 73, wherein the target mRNA is mRNA responsible for apoptosis development~~, and wherein the quantification of mRNA is used to test the side effects of anti cancer drugs that induce mRNA responsible for apoptosis development~~.

234. **(Withdrawn)** The method of Claim 73, wherein the mRNA of DNA-repair genes is quantified.

235. **(Withdrawn)** The method of Claim 234, wherein the quantification of mRNA of DNA-repair genes is used to test the sensitivity of DNA-repair genes to radiation.

236. **(Withdrawn)** The method of Claim 73, wherein the mRNA of allergen response genes is quantified.

237. **(Withdrawn)** The method of Claim 236, wherein the quantification of mRNA of allergen response genes is used to test allergen stimulation.

238. **(Canceled)**

239. **(Canceled)**

240. **(Previously Presented)** The method of Claim 217, wherein each of said different mRNAs is amplified from the cDNA formed by extension of the immobilized oligo(dT) in step (g).

SUMMARY OF INTERVIEW

Attendees, Date and Type of Interview

A personal interview was conducted on October 28, 2009 at the USPTO and attended by Examiner Lu, Masato Mitsuhashi, Jason Gersting, and Daniel Altman.

Exhibits and/or Demonstrations

N/A

Identification of Claims Discussed

Claims 73, 215, 218, and 233

Proposed Amendments

Applicant proposed to amend Claim 73 to more specifically define the identity of the target mRNA. Applicant proposed to amend Claims 215 and 218 to clarify that quantitation of the target mRNA involved amplification. Applicant proposed to Amend Claim 233 to address the new matter rejection.

Principal Arguments and Other Matters

Applicant explained certain aspects of the invention to the Examiner, in particular how reverse transcriptase can create at least two molecules of cDNA from a single RNA template using both the bound oligo-dT and one or more specific primers as sites from which reverse transcription may begin (as depicted in Figure 15). Applicant further explained that the results shown in Figure 16 show that the cDNA resulting from extension of the oligo-dT primer removes the cDNA produced from extension of the added primer, thereby eliminating the need for heat denaturation. Applicant further discussed with Examiner Lu the rejection of portions of the previously filed Preliminary Amendment as containing new matter. Applicant indicated examples of relevant passages of the specification as filed and the drawings that support the Amendment.

Results of Interview

Examiner Lu accepted Applicant's explanation with respect to the mechanism of displacement of cDNA strands without heat denaturation as well as the lack of new matter in the

previously filed Preliminary Amendment. The Examiner also indicated that the proposed amendments appeared acceptable and that remarks indicating the passages of the specification supporting the material included in the previously filed Preliminary Amendment, as well as the mechanisms of cDNA displacement should be identified in Applicant's next response.